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Attorney's Docket No. U 011415-0

CHAPTER II

IN THE UNITED STATES ELECTED OFFICE (EO/US)

PCT/AU96/00149	15 MARCH 1996	16 MARCH 1995
INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY CLAIMED

ANTIGEN COMPOSITION AGAINST MYCOPLASMA

TITLE OF INVENTION

1. JOHN WALKER
2. ROGAN LEE

APPLICANT(S)

3. STEPHEN WILLIAM DOUGHTY

Box PCT
Commissioner of Patents and Trademarks
Washington, D.C. 20231
ATTENTION: EO/US

COMPLETION OF FILING REQUIREMENTS FOR INTERNATIONAL APPLICATION ENTERING U.S. NATIONAL STAGE IN U.S. ELECTED OFFICE (EO/US) UNDER 35 USC 371

(check and complete the applicable item, if applicable)

☒ This replies to the Notice of Missing Requirements under 35 U.S.C. 371 and 37 CFR 1.495 (FORM PCT/DO/EO/905).

☒ A copy of FORM PCT/DO/EO/905 accompanies this response.

WARNING: *Where the items being submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 CFR 1.10 must be used (because international application papers are not covered by an ordinary certificate of mailing 37 CFR 108(2)(xi)*

NOTE Documents and fees must be clearly identified as a submission to enter the national stage under 35 USC 371. Otherwise, the submission will be considered as being made under 35 USC 111 37 CFR 1.495(g).

CERTIFICATE UNDER 37 CFR 1.10

I hereby certify that this Completion of Filing Requirements and the papers indicated as being transmitted therewith are being deposited with the United States Postal Service on this date Dec. 9, 1997, in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EI528037809US, addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Geraldine Marti
(type or print name of person mailing paper)

Geraldine Marti
Signature of person mailing paper

NOTE Each paper referred to as enclosed herein has the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 CFR 1.16(b)

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 CFR 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence*

EI528037809US

DECLARATION OR OATH

I. ☒ No original declaration or oath was filed. Enclosed is the original declaration or oath for this application.

NOTE: For surcharge fee for filing declaration after filing date complete item IV(3)

NOTE: Acceptable minimums in the declaration in an ordinarily filed U.S. application for identification of the specification to which it applies are the name of the inventor and (1) serial number, (2) attorney docket number that was on the application as filed and the filing date, (3) title of the invention and filing date, (4) title of invention and reference to a specification that is attached to the declaration at the time of execution and filed with the declaration, or (5) title of invention and a statement by a registered attorney that the application filed in the PTO is the application which the inventor executed by signing the declaration. If the identification (4) is used it must be accompanied by a statement that the "attached" specification is a copy of the specification and any amendments thereto that were filed in the PTO to obtain the filing date. Such a statement must be a verified statement if made by a person not registered to practice before the PTO. Notice of September 12, 1983 (1035 O.G. 3)

NOTE: Another minimum found acceptable in the declaration is the filing date (i.e., date of express mail) and the express mail number, useful where the serial number is not yet known. But note the practice where the express mail deposit is a Saturday, Sunday or holiday within the District of Columbia. 37 CFR 1.10(c)

NOTE: 37 CFR 1.41(a) points out that "Full names must be stated, including the family name and at least one given name without abbreviation together with any other given name or initial."

(complete (c) or (d), if applicable)

Attached is a

(c) ☐ Statement by a registered attorney that the application filed in the PTO is the application that the inventor executed by signing the declaration.

(d) ☐ Statement that the "attached" specification is a copy of the specification and any amendments thereto that were filed in the PTO to obtain the filing date.

AMENDMENT

II. *(complete as applicable)*

☐ An amendment in accordance with 37 CFR § 1.121 is attached.

☐ The attached amendment cancels claims _____ inclusive.

TRANSMITTAL OF ENGLISH TRANSLATION OF NON-ENGLISH LANGUAGE PAPERS

III. ☐ Submitted herewith is a English translation of the non-English language international application papers as originally filed. It is requested that this translation be used as the copy for examination purposes in the PTO. (See 37 CFR 1.495(c))

NOTE: For fee for processing a non-English application, complete item IV(4)

NOTE: A non-English oath or declaration in the form provided or approved by the PTO need not be translated 37 CFR 1.69(b)

NOTE: Unlike the filing of an ordinary non-English application (37 CFR 1.52(d)), the translation of an international application entering the U.S. national phase need not be verified. 37 CFR 1.495(c). If necessary, however, a verified translation may be required. 37 CFR 1.495(c). Moreover, if the English translation is filed within 30 months from the priority date, no processing fee is required

FEES

IV.

NOTE. The fees for claims and surcharge fees listed below in items 1 and 2 are reduced by 50% where proof of a small entity status is established on or before the date the fee is paid. If the full fee was paid, but a verified statement is filed within 2 months of the date of timely payment of a fee, then the excess fee paid will be refunded on request. 37 CFR 1.28(a).

1. Fees for claims
   - ☐ each independent claim in excess of 3 (37 CFR 1.492(b))—$78.00 small entity—$39.00 $_____
   - ☐ each claim in excess of 20 (37 CFR 1.492(c))—$22.00; small entity—$11.00 $_____
   - ☐ multiple dependent claims(s) (37 CFR 1.492(d))—$250.00 small entity—$125.00 $_____
2. Surcharge fees
   - ☐ surcharge set forth in 37 CFR 1.492(e) for accepting the declaration later than 30 months after the priority date in filing an application in the U.S. as a designated office—$130.00; small entity—$65.00 $_____

NOTE The processing fee in the next item 3 below is not subject to a reduction for small entity status

3. ☒ processing fee set forth in 37 CFR 1.492(f) for acceptance of an English translation later than 30 months after the priority date—$130.00 $ 130.00

Total fees $_____

SMALL ENTITY STATUS

V. ☐ A verified statement that this filing is by a small entity

NOTE If an original verified statement and a refund request are filed within two months of the date of payment of a fee, then the excess fee paid will be refunded on request. 37 CFR 1.28(a)

(check and complete applicable items)

- ☐ is attached.
  - ☐ A separate refund request accompanies this paper.

EXTENSION OF TIME

(complete (a) or (b), as applicable)

VI.

The proceedings herein are for a patent application. Accordingly, the provisions of 37 CFR § 1.136(a) apply.

(a) ☐ Applicant petitions for an extension of time, the fees for which are set out in 37 CFR § 1.17(a)-(d), for the total number of months checked out below:

Extension (months)	Fee for other than small entity	Fee for small entity
☐ one month	$ 110.00	$ 55.00
☐ two months	$ 380.00	$ 190.00
☐ three months	$ 900.00	$ 450.00
☐ four months	$1,400.00	$ 700.00

Fee: $ _____

If an additional extension of time is required, please consider this a petition therefor.

(check and complete the next item, if applicable)

☐ An extension for _____ months has already been secured, and the fee paid therefor of $ _____ is deducted from the total fee due for the total months of extension now requested.

Extension fee due with this request $_____

or

(b) ☒ Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

TOTAL FEE DUE

VII.

The total fee due is:

Completion fee(s) $ 130.00

Extension fee (if any) $_____

TOTAL FEE DUE $ 130.00

PAYMENT OF FEES

VIII.

☒ Enclosed is a check in the amount of $ 130.00.

☐ Charge Account No. _____ in the amount of $_____.

☐ A duplicate of this request is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b)

AUTHORIZATION TO CHARGE ADDITIONAL FEES

IX.

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authonzed*

WARNING: *"Submission of the appropnate extension fee under CFR 1.136(a) is to no avail unless a request or petition for extension is filed." Notice of November 5, 1985 (1060 O.G 27)*

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 12-0425

☒ 37 CFR 1.492(a)(1), 1.492(a)(4) (filing fees)

☐ 37 CFR 1.492(b), (c), and (d) (presentation of extra claims)

NOTE. Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time penod set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authonze the PTO to charge additional claim fees, except possibly when dealing with amendments after final action

☒ 37 CFR 1.17 (application processing fees)

WARNING: *While 37 CFR 1.17(a), (b), (c) and (d) deal with extensions of time under § 1 136(a) this authonzation should be made only with the knowledge that "Submission of the appropnate extension fee under 37 C.F.R 1.136(a) is to no avail unless a request or petition for extension is filed " (Emphasis added) Notice of November 5, 1985 (1060 O.G. 27)*

☐ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b).

NOTE Where an authonzation to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b)

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . pnor to paying, or at the time of paying . issue fee " From the wording of 37 CFR 1.28(b) (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity

☐ 37 CFR 1.492(e) and/or (f) surcharge fees for filing the declaration and/or an English translation of an international application later than 30 months from the earliest-claimed priority date.

WARNING: *It would be wise to always check this last authonzation.*

SIGNATURE OF ATTORNEY

Reg. No.:

Tel. No.: ( )

(type or print name of attorney) Clifford J. Mass
Registration No. 30,086
c/o LADAS & PARRY
26 West 61st Street
P.O. Address New York, NY 10023
(212) 708-1890

Attorney's Docket No. U 011415-0

CHAPTER II

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU96/00149	15 MARCH 1996	16 MARCH 1995

TITLE OF INVENTION: ANTIGEN COMPOSITION AGAINST MYCOPLASMA

APPLICANT(S):
1. JOHN WALKER
2. ROGAN LEE
3. STEPHEN WILLIAM DOUGHTY

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

ATTENTION: EO/US

NOTE: The completion of those filing requirements that can be made at a time later than 30 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 USC 371(d). The filing receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 CFR 1.491 which states: "An international application enters the national state when the applicant has filed the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495."

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 CFR 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - 37 CFR 1.8 (2) (xi)).*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 CFR 1.494(f).

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date SEPT. 12, 1997, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EH684275215, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

GERALDINE MARTI

(type or print name of person mailing paper)

Geraldine Marti

Signature of person mailing paper

NOTE: Each paper or fee referred to as enclosed herein has the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 CFR 1.16(b).

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 CFR 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 CFR 1.492) as indicated below:

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS	
☐*	TOTAL CLAIMS	43 -20=	23	× $22.00 =	$ 506.00	NOT PAID AT THIS TIME
	INDEPENDENT CLAIMS	8 -3=	5	× $80.00=	400.00	NOT PAID AT THIS TIME
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$260.00		
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4)) ..... $96.00 ☐ and the above requirements are not met (37 CFR 1.492(a)(1)) ..... $700.00 ☒ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 CFR 1.492(a)(2)) ..... $770.00 ☒ has not been paid (37 CFR 1.492(a)(3)) ..... $1040.00 ☐ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) $910.00					
	Total of above Calculations				= 1,040.00	
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 CFR 1.9, 1.27, 1.28)				-	
	Subtotal					
	Total National Fee				$ 1,040.00	
	Fee for recording the enclosed assignment document $40.00 (37 CFR 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".					
TOTAL	Total Fees enclosed				$ 1,040.00	

*See attached Preliminary Amendment Reducing the Number of Claims.

i. ☒ A check in the amount of 1,040.00 to cover the above fees is enclosed.

ii. ☐ Please charge Account No. _____ in the amount of $ _____. A duplicate copy of this sheet is enclosed.

***WARNING:** *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 CFR § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 CFR § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of January 3, 1993, 1147 O.G. 29 to 40.*

3. ☒ A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of January 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☐ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☒ has been transmitted

i. ☒ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308): _____.

ii. ☐ by applicant on (date) _____.

4. ☒ A translation of the International application into the English language (35 U.S.C. 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on (*date*) _____.

d. ☐ will follow.

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 CFR § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. ☐ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308): _____.

ii. ☐ by applicant on (date) _____.

c. ☒ have not been transmitted as

i. ☒ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.): _____.

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☐ is not required as the amendments were made in the English language.

c. ☒ has not been transmitted for reasons indicated at point 5c above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)

☒ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☐ Annex(es) to the international preliminary examination report

a. ☐ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☐ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115

a. ☐ was previously submitted by applicant on (date) _____.

b. ☐ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3b or 3c and 5b; and states that they were reviewed by the inventor as required by 37 CFR 1.70.

iii. ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☒ is transmitted herewith.

b. ☐ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on (date) _____.

12. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98:

a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

☐ Form PTO-1449.

☐ Copies of citations listed.

b. ☒ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).

c. ☐ was previously submitted by applicant on (date) _____.

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

_____

_____

_____

14. ☒ Additional documents:

a. ☐ Copy of request (PCT/RO/101)

b. ☒ International Publication No. WO 96/28472

i. ☒ Specification, claims and drawing

ii. ☐ Front page only

c. ☐ Preliminary amendment (37 CFR § 1.121)

d. ☒ Other

FORM PCT/IPEA/402

15. ☒ The above checked items are being transmitted

a. ☒ before 30 months from any claimed priority date.

b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 12-0425

☒ 37 CFR 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 CFR § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☐ 37 CFR 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☒ 37 CFR 1.17 (application processing fees)

WARNING: *While 37 CFR 1.17(a), (b), (c) and (d) deal with extensions of time under § 1.136(a), this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 CFR 1.136(a) is to no avail unless a request or petition for extension is filed." Notice of November 5, 1985 (1060 O.G. 27).*

☐ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☒ 37 CFR 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF ATTORNEY Clifford J. Mass

Reg. No.: Registration No. 30,086

Tel. No.: ( ) c/o LADAS & PARRY

(type or print name of attorney) 26 West 61st Street

New York, NY 10023

(212) 708-1890

P.O. Address

08/913430

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: John WALKER, et al

Serial No.: 08/913,430 Group No: ---

Filed: September 12, 1997 Examiner: ---

For: ANTIGEN COMPOSITION AGAINST MYCOPLASMA

Attorney Docket No.: U-011415-0

Commissioner Patents and Trademarks
Washington, DC 20231

AMENDMENT

Sir:

Prior to an examination of this application on the merits, please amend the application as follows:

IN THE CLAIMS:

Please cancel claims 1 - 43 and replace with the following new claims.

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner of Patents and Trademarks, Washington, DC 20231

CLIFFORD J. MASS
(Type or print name of person mailing paper)

Date: September 30, 1997

(Signature of person mailing paper)

44. A putative protective antigen against a Mycoplasma, prepared by a method including

providing

a sample of a Mycoplasma;

an antibody probe including at least antibody against a Mycoplasma produced by a method including

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in suitable culture medium; and

harvesting antibodies produced from said cells;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

45. A putative protective antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), mutants, derivatives and fragments thereof.

46. A putative protective antigen according to claim 45 wherein the antigen in the 72-75 kD region contains the following N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

47. A putative protective antigen according to claim 46 further including one or more of the following internal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLDN

48. A putative protective antigen according to claim 45 wherein the antigen in the 60-64 kD region contains the following N-terminal amino acid sequence:

MKLAKLLKGFX(N/L)(M/V)IK, or

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

49. A putative protective antigen according to claim 45 wherein the antigen in the 52-54 kD region contains the following N-terminal amino acid sequence:

AGXWAKETTKEEKS

50. A putative protective antigen according to claim 49 further including one or more of the following internal amino acid sequences:

AWVTADGTVN

AIVTADGTVNDNKPNQWVRKY

51. A putative protective antigen according to claim 45 wherein the antigen in the 46-48 kD region contains the following N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

52. A putative protective antigen according to claim 51 further including one or more of the following internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR

AEQAITKLKLEGFDTQ

KNSQNKIIDLSPEG

53. An isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment including the following sequence, mutants, derivatives, recombinants and fragments thereof:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550
TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782

54. A method for producing an antibody against a Mycoplasma including providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells.

55. A method according to claim 54 wherein the biological sample is taken approximately 2 to 7 days after the animal has been challenged with the Mycoplasma.

56. A method according to claim 55 wherein the culturing of cells in vitro further includes addition of helper factors to the culture, said helper factors selected from the group including cytokines used alone or in combination, including interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.

57. A method according to claim 56 further including a cell activation step including activating the cells isolated to proliferate and secrete and/or release antibodies, said cell activation step including adding a cell activating agent to the culture medium, said cell activating agent selected from the group including mitogens and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.

58. A method according to claim 57 wherein the antibody is in the form of the supernatant harvested from the culture medium.

59. An antibody against a Mycoplasma prepared according to the method of claim 54.

60. A method of identifying a putative protective antigen associated with a Mycoplasma, said method including

providing

a sample of a Mycoplasma; and

an antibody probe including at least one antibody against a

Mycoplasma;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

61. A method of purifying a putative protective antigen associated with a Mycoplasma, said method including providing

a crude antigen mixture; and

an antibody against a Mycoplasma immobilized on a suitable support;

subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and

isolating the purified antigen so formed.

62. A method for preparing a synthetic antigenic polypeptide against Mycoplasma, which method includes

providing

a cDNA library or genomic library derived from a sample of Mycoplasma; and

an antibody probe including an antibody prepared according to claim 54;

generating synthetic polypeptides from the cDNA library or genomic library;

probing the synthetic polypeptides with the antibody probe; and

isolating the synthetic antigenic polypeptide detected thereby.

63. A method according to claim 62 wherein the antibody probe includes an antibody raised against an antigen against Mycoplasma hyopneumoniae, or related infections selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), mutants, derivatives and fragments thereof.

64. A synthetic putative protective antigen produced by the method of claim 62.

65. A vaccine or veterinary composition including a prophylactically effective amount of at least one putative protective antigen against a Mycoplasma according to claim 45.

66. A vaccine or veterinary composition according to claim 65 including a plurality of putative protective antigens.

67. A vaccine or veterinary composition including an antibody against a Mycoplasma according to claim 59.

68. A diagnostic kit including an antigen according to claim 45.

69. A method for preventing or treating a Mycoplasma infection, which method including administering to an animal a prophylactically or therapeutically effective amount of at least one putative protective antigen according to claim 45.

70. An isolated DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragment having a nucleic acid sequence according to Figure 6 or an homologous sequence, and functionally active fragments, mutants, variants or recombinants thereof.

71. A clone including a DNA fragment according to claim 70.

72. A clone according to claim 71 which is clone pC1-2

73. An amino acid sequence or functional equivalent thereof encoded by the DNA fragment according to claim 70.

74. An amino acid sequence or functional equivalent thereof having an amino acid sequence according to Figure 7.

REMARKS

Claims 1 - 43 have been cancelled and replaced with new claims 44 - 74. The recitations in claims 44 - 74 correspond to recitations in the claims originally filed as shown on the marked-up copy of the original claims annexed hereto. Accordingly, the new claims draw clear support from the specification as filed.

An early examination of the application is now respectfully requested.

Respectfully submitted,

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ANTIGEN COMPOSITION AGAINST MYCOPLASMA

The present invention relates to protective and diagnostic antigens, the preparation thereof, and their use in the formation of vaccine compositions, particularly vaccine compositions against Mycoplasma hyopneumoniae infections.

Mycoplasma hyopneumoniae is a ubiquitous swine respiratory pathogen causing mycoplasmal pneumoniae in swine (swine enzootic pneumonia). Swine enzootic pneumonia is probably the most widespread and economically significant disease in swine producing countries of the world. The economic effects of swine enzootic pneumonia (SEP) are complex, and the cost of the disease is severe. In Australia, the disease was estimated in 1988 to cost approximately $20,000,000 per annum. Increased mortality, decreased growth weight, depressed feed conversion, susceptibility to secondary bacterial infections, increased management costs, and increased use of antibiotics, are the main reasons for the economic impact of SEP.

Whilst several experimental vaccines have been produced, these have resulted in less than optimal results, and utilising various classes of antibiotics such as tetracycline, lincamycin and tiamulin is still the most widespread control treatment. Such antibiotics are, however of limited therapeutic value, because they do not prevent the establishment of an infection and lung lesions may develop after treatment ends.

European Patent Application 359 919 to ML Technology Ventures L.P. describes a series of antigens, 36 kD, 41 kD 74.5 kD and 95 kD in size, and proposes the use of such antigens in vaccines. Results presented suggest that some protection in pigs against challenge was achieved.

However, there remains a need in the art for an effective vaccine against M. hyopneumoniae which would confer protection against colonisation and clinical disease following M. hyopneumoniae challenge and also significantly reduce the morbidity and mortality from secondary infections

Accordingly, it is an object of the present invention to overcome, or at least alleviate, one or more of the difficulties and deficiencies in the prior art

Accordingly, in a first aspect of the present invention there is provided a putative protective antigen against a Mycoplasma, preferably Mycoplasma

hyopneumoniae prepared by a method including

providing

a sample of a Mycoplasma;

an antibody probe including at least one antibody against a Mycoplasma produced by a method including;

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

The protective antigens may also function as diagnostic antigens as discussed below.

Accordingly, in a preferred aspect of the present invention there is provided a putative protective antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), as hereinafter described, mutants, derivatives and fragments thereof. The putative protective antigen may be a surface protein. The putative protective antigen may be a surface lipoprotein or membrane protein.

Preferably the protective antigens are selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 74, 62, 52 and 48 kD.

Preferably, the 72-75 kD antigen includes the following N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

and, optionally, one or more of the following internal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLDN

Preferably, the 60-64 kD antigen includes one of the following N-terminal amino acid sequences:

MKLAKLLKGFX(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

Preferably, the 52-54 kD antigen includes the following N-terminal amino acid sequence:

AGXWAKETTKEEKS

and, optionally, one or more of the following internal amino acid sequences:

AWVTADGTVN

AIVTADGTVNDNKPNQWVRKY.

Preferably, the 46-48 kD antigen includes the following N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

and, optionally, one or more of the following internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR

AEQAITKLKLEGFDTQ

KNSQNKIIDLSPEG

The 46-48 kD antigen may be encoded by a nucleic acid fragment:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900

	ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
	TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
	ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
	GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
5	AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
	CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
	CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
	TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
	GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
10	AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
	TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
	TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
	GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550
	TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
15	TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
	AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
	AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
	CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782

Accordingly, in a further aspect the present invention provides an isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350

AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550
TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782

As cross protection between various Mycoplasma such as M. hyorhinis and M. synoviae has been documented, similar antigens may also be detected in other Mycoplasma species (Figure 1).

In a still further aspect the present invention provides a method for preventing Mycoplasma infection in animals. Preferably the Mycoplasma disease is a Mycoplasma hyopneumoniae disease such as swine enzootic pneumonia (SEP). This method includes administering to an animal an effective amount of at least one protective antigen against Mycoplasma as described above.

The present invention further provides a vaccine composition including a prophylactically effective amount of at least one putative protective antigen against a Mycoplasma as herein described. Preferably the veterinary composition includes two or more putative protective antigens as herein described.

Accordingly in a preferred aspect the present invention provides a vaccine composition including two or more putative protective antigens selected from antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons.

The vaccine composition may include any combination of two or more putative protective antigens selected from antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kD. The two or more antigens may be selected from antigens falling within one of the specified approximate molecular weights and/or antigens from different specified approximate molecular weights. The composition may contain 3, 4, 5 or 6 antigens selected from protective antigens having molecular weights of approximately 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kD.

The vaccine compositions according to the present invention may be

administered orally or may be administered parenterally (for example by intramuscular, subcutaneous, intradermal or intravenous injection). The amount required will vary with the antigenicity of the active ingredient and need only be an amount sufficient to induce an immune response typical of existing vaccines.

Reactive experimentation will easily establish the required amount. Typical initial doses of vaccine or veterinary compositions may be approximately 0.001-1 mg active ingredient/kg body weight. The dose rate may increase or multiple doses may be used as needed to provide the desired level of protection.

The vaccine composition according to the present invention may further include a veterinary acceptable carrier, diluent or excipient therefor. Preferably the active ingredient may be suspended or dissolved in a carrier. The carrier may be any solid or solvent that is nontoxic to the animal and compatible with the active ingredient. Suitable carriers include liquid carriers, such as normal saline and other nontoxic salts at or near physiological concentrations, and solid carriers, such as talc or sucrose.

Preferably the vaccine contains an adjuvant, such as Freund's adjuvant, complete or incomplete, or immunomodulators such as cytokines may be added to enhance the antigenicity of the antigen if desired.

More preferably the adjuvant is of the mineral-oil type as these have been found to be consistently superior at inducing antibody titres and Delayed Type Hypersensitivity responses. A particularly preferred adjuvant is that marketed under the trade designation Montanide ISA-50 and available from Seppic, Paris, France.

When used for administering via the bronchial tubes, the vaccine is suitably present in the form of an aerosol.

In a still further aspect of the present invention there is provided a diagnostic kit including a diagnostic antigen against a Mycoplasma, preferably Mycoplasma hyopneumoniae, identified and purified as described above.

The putative protective antigens according to the present invention may be isolated and identified utilising the general methods described in Australian patent application 49035/90, the entire disclosure of which is incorporated herein by reference

Accordingly, in a further aspect, the present invention provides a method for producing at least one antibody against a Mycoplasma. This method includes

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells.

The Mycoplasma may be Mycoplasma hyopneumoniae.

The animal may be a mammal including humans. The mammal may be a domestic animal such as a pig, sheep or cattle.

The biological animal sample may be of any suitable type. The biological sample may be taken from animal tissue, organs, lymph or lymph nodes. The biological sample may be taken from the infection site, the lungs of the animal, or an area of a lesion which may be formed or an area close to the infected site or a lesion such as in the lymph nodes draining from the lungs.

However, serum/plasma samples are not used as the biological samples according to this aspect of the present invention. It has been found that the majority of antibodies found in a serum/plasma sample are irrelevant to protection or specific diagnosis or a Mycoplasma or are unrelated to the Mycoplasma. In addition, other serum/ plasma components may interfere with the specific reactions between pathogen components and antibodies to them.

In contrast, the probes described in the present invention are highly enriched in Mycoplasma-specific antibodies of particular importance to protective immunity.

It is preferred that the biological samples are taken from the animals at a predetermined time in the development of the disease. In general, for a Mycoplasma infection, it has been found that the biological samples should be taken approximately 2 to 7 days after challenge with or after administration of products obtained from a pathogen or with the pathogen itself.

The cells isolated from the biological sample may include B cells.

Thus, preferably the cells are taken a short time after in vivo stimulation.

preferably within approximately 2 to 5 days thereafter, resulting in the in vivo induction of antibody forming cells which will secrete specific antibodies into the culture medium after in vitro incubation.

In vitro secretion of antibodies in the culture medium by recently activated B cells may be enhanced by the addition of helper factors to the cultures. The helper factors may be cytokines used alone or in combination, including Interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.

The method of producing an antibody may include a further step of activating the cells isolated to proliferate and secrete and/or release antibodies.

The cell activation step may include adding a cell activating agent to the culture medium. The cell activating agent may be selected from mitogens and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.

The mitogens may be selected from the group including products derived from pokeweed (Phytolacca americana) also known as pokeweed mitogen (PWM), polyvinylpyrrolidone (PVP), polyadenylic-polyuridylic acid (poly(A-U)), purified protein derivate (PPD), polyinosinic-polycytidilic acid (poly(I-C)), lipopolysaccharide (LPS), staphylococcal organisms or products thereof, Bacto-streptolysin O reagent (SLO), Staphylococcal phage lysate (SPL), Epstein-Barr virus (EBV), Nocardia water-soluble mitogen (NWEM), phytohemagglutinin (PHA), Concanavalin A (Con A), and dextran-sulphate and mixtures thereof. The cell proliferation agent may be any agent that indirectly or directly results in B cell proliferation and/or antibody secretion such as solid-phase anti-immunoglobulin. The helper factors may be selected from the group including cytokines including interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other helper factors that may be shown when added alone, or in combination with other factors and agents, to have an enhancing effect on specific B cell proliferation and/or antibody secretion. This in no way is meant to be an exhaustive list of mitogens and cell activating agents including helper factors.

The in vitro culturing of the cells may be conducted with or without prior

steps to separate sub-populations of cells. The harvesting of antibodies may be conducted by harvesting of the supernatant from the culture medium. This supernatant contains antibodies secreted by these cells during the in vitro culture or artificially released from the B cells, for example by lysis of the B cells. It has been found that the antibody-containing supernatants may be used directly to detect antigens of the Mycoplasma.

In a preferred aspect of the present invention, there is provided a method for identifying an antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae. This method includes

providing

a sample of a Mycoplasma; and

an antibody probe including at least one antibody against a Mycoplasma;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

The sample of Mycoplasma may be mixed with a standard buffer solution and placed on a standard support such as an SDS-polyacrylamide gel to separate the proteins contained thereon (Figure 2)

Alternatively the proteins may be selected utilising the non-ionic detergent Triton X-114 (TX-114). Insoluble material may be removed by centrifugation. Proteins soluble in the TX-114 phase may then be precipitated out (Figure 2).

The separate proteins may then be transferred to nitrocellulose, nylon or other sheets.

The probing with a suitable antibody may further include subjecting the product produced thereby to a detection assay. The detection assay may include Western blot techniques. The detection assay may be an immunoprecipitation assay a radioimmunoassay, an enzyme-linked immunoassay or immunofluorescent assay (Figures 3, 4 and 5)

The antibody produced as described above may be utilized simply in the form of the supernatant harvested from the culture medium. Alternatively, the antibodies may be separated and purified

In a further preferred aspect of the present invention the antibody contained in the culture medium may be used for the affinity purification, preferably immuno-affinity purification of antigen.

Accordingly, in a preferred aspect there is provided a method for purifying antigen. This method includes

providing

a crude antigen mixture; and

an antibody against a Mycoplasma immobilized on a suitable support;

subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and

isolating the purified antigen so formed.

The antibody is produced by the method described above.

Antibody can be obtained from the culture supernatant probe by conventional methods. For example, methods usually used to purify immunoglobulins from serum or plasma, e.g. precipitation with ammonium sulphate, fractionation with caprylic acid, ion exchange chromatography, or by binding and elution from immobilized protein G or protein A, may be utilized. Antibody so obtained can then be coupled to suitable supports, e.g., CNBr-activated Sepharose 4B (Pharmacia), Affi-gel (Bio-RAD), or other affinity chromatography supports able to bind proteins.

Immobilized antibody can then be applied to the fractionation and purification of specific antigen from a complex Mycoplasma extract by affinity chromatography. After binding of antigen to immobilized antibody, unbound macromolecular species can be washed away from the solid support with, e.g buffers containing 1.5 M NaCl Subsequently the antigen can be eluted from the affinity column with, e.g. low or high pH buffer or buffers containing chaotropic ions, e.g. 0.5-3.0 M sodium thiocyanate.

The application of the antibody probe to affinity chromatography enables sufficient quantities of specific antigens to be rapidly isolated from a complex crude extraction mixture for biochemical characterization, amino-acid sequencing and vaccination of animal for limited protection studies. Application of affinity

chromatography for obtaining antigen(s) avoids the difficulties often encountered when applying conventional biochemical techniques to the purification of an antigen about which little or no data is known. It also obviates the need to raise polyclonal or monoclonal antibodies for the purpose of "analytical" affinity chromatography. Large scale preparation may, however, require the preparation of polyclonal or monoclonal antibodies.

Having identified the antigen(s) molecular biology, chemical techniques, e.g. cloning techniques, may be used to produce unlimited amounts of this antigen or, alternatively, synthetic peptides corresponding to different fragments of the identified antigens may be used as a means to produce a vaccine.

Accordingly in a preferred aspect of the present invention there is provided a method for preparing a synthetic antigenic polypeptide against Mycoplasma, preferably Mycoplasma hyopneumoniae, which method includes

providing

a cDNA library or genomic library derived from a sample of Mycoplasma; and

an antibody probe as described above;

generating synthetic polypeptides from the cDNA library or genomic library;

probing the synthetic polypeptides with the antibody probe; and

isolating the synthetic antigenic polypeptide detected thereby.

Either cDNA or genomic libraries may be used. The cDNA or genomic libraries may be assembled into suitable expression vectors that will enable transcription and the subsequent expression of the clone cDNA, either in prokaryotic hosts (e.g. bacteria) or eukaryotic hosts (e.g. mammalian cells). The probes may preferably be selected from

(i) synthetic oligonucleotide probes based on the amino acid sequence of the antigen identified and purified as described above.

(ii) antibodies obtained from the culture medium produced as described above;

(iii) monoclonal or polyclonal antibodies produced against the antigens identified and purified as described above;

(iv) recombinant or synthetic monoclonal antibodies or polypeptides with

specificity for the antigen, e.g. as described by Ward et al., Nature, 241, pages 544-546 (1989).

The synthetic antigenic polypeptide produced in accordance with the invention may be a fusion protein containing the synthetic antigenic peptide and another protein.

In a further aspect of the present invention there is provided a DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragments having a nucleic acid sequence according to Figure 6a and 6b or an homologous sequence and functionally active fragments thereof.

In a further preferred aspect of the present invention there is provided a clone including a DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragments having a nucleic acid sequence according to Figure 6a and 6b or an homologous sequence and functionally active fragments thereof.

Preferably the clone is pC1-2.

The present invention will now be more fully described with reference to the accompanying Examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

IN THE FIGURES:

FIGURE 1: SDS-Polyacrylamide gel (12.5%) profiles of SDS extracts of species of mycoplasma- Coomassie R250 stained.

Lane 1 Pre-stained Molecular Weight Standards

Lane 2 *M. gallisepticum*

Lane 3 *M. synoviae.*

Lane 4 *M. hyopneumoniae.*

Lane 5 *M. hyorhinis.*

Lane 6 *M. flocculare.*

FIGURE 2: SDS-Polyacrylamide gel (12.5%) profiles of extracts of strains *of M. hyopneumoniae* - Coomassie R250 stained gel

Lane 1 Pre-stained Molecular Weight Standards.

Lane 2 Triton X-114 extract of *M. hyopneumoniae* - strain Beaufort.

Lane 3 As for Lane 2.

Lane 4 SDS extract of *M. hyopneumoniae* strain Beaufort.

Lane 5 SDS extract of *M. hyopneumoniae* strain 10110.

FIGURE 3: Western blots of Triton X-114 extracted antigens from *M. hyopneumoniae* strain Beaufort, probed with serum and supernatant antibody probes.

Lane 1 No antibody control

Lane 2 Dookie pig serum control 1/200.

Lane 3 Pig 105 supernatant.

Lane 4 Pig 1 supernatant.

Lane 5 Dookie pig supernatant.

FIGURE 4: Western blots of SDS extracted antigens from *M. hyopneumoniae* strain Beaufort probed with paired serum and supernatantantibody probes. Fractionation of antigens on SDS Polyacrylamide gel (12.5%).

Lane 1 a) Pig 453 supernatant.

b) Pig 453 serum 1/100.

Lane 2 a) Pig 105 supernanant.

b) Pig 105 serum 1/100.

Lane 3 a) Pig 1 superanatant.
b) Pig 1 serum 1/100.
Lane 4 a) Pig 15 supernanant.
b) Pig 15 serum 1/100.
Lane 5 a) Dookie supernatant.
b) Dookie serum 1/100.
Lane 6 No antibody control.

FIGURE 5: Western blots of SDS extracted antigens from *M. hyopneumoniae* strain Beaufort probed with paired serum and supernatantantibody probes. Fractionation of antigens on SDS Polyacrylamide gel (10.0 %).

Lane 1 a) Pig 453 supernatant.
b) Pig 453 serum 1/100.
Lane 2 a) Pig 105 supernatant.
b) Pig 105 serum 1/100.
Lane 3 a) Pig 1 supernatant.
b) Pig 1 serum 1/100.
Lane 4 a) Pig 15 supernatant.
b) Pig 15 serum 1/100.
Lane 5 a) Dookie supernatant.
b) Dookie serum 1/100.
Lane 6 No antibody control.

FIGURE 6: The entire 48 k gene sequence.

FIGURE 7: tHE 48kDa protein sequence of the 48k gene sequence.

EXAMPLE 1

Mycoplasma hyopneumoniae media

Friss Media

Hovind-Hougen, K., Friss, N.F., Research in Veterinary Science, 1991, 51, pp 155-163, "Morphological & Ultrastructural Studies of M flocculare and M hyopneumoniae in vitro".

250 ml Hanks BSS
140 ml Water
1.5 gm Brain Heart infusion
1.6 gm PPLO Broth w/o CV
Autoclave at 120°C for 20 minutes
18 ml Yeast Extract (100g YSC-2 Sigma in 750 ml)
3.7 ml 0.2% DNA in 0.1% Na_2CL_3
5.14 ml 1% -NAD
0.6 ml 1% Phenol red

Adjust to pH 7.3 to 7.4
Filter through 0.45 um, 0.2 um membrane, store at 4°C.
Add sterile Horse or Pig serum to 20%
and Antibiotics prior to use

Etheridge Media

Etheridge, J.R., Cottew, G.S., Lloyd, L.C., Australian Veterinary Journal, 1979, August 55, pp 356-359, "Isolation of Mycoplasma hyopneumoniae from lesions in experimentally infected pigs".

Materials	For 600 mls
Hanks BSS	18.9 ml
Hartleys Digest broth	1.28 gm
Heart Infusion broth	1.65 gm
Lactalbumin hydrolysate	2.21 gm
Glucose	4.41 gm
Yeast Extract autolysate	8.82 ml
Pig Serum (filtered)	163 ml
1% NAD	6.17 ml
1% Phenol red	1.32 ml
0.2% DNA in 0.1% Na_2CO_3	4.41 ml

Make up to 600 ml with MQ water (about 350 - 400 ml)

Adjust pH to 7.4 and filter through: 3.0 um, 0.8 um 0.45 um, 0.2 um.

Store at 4°C.

Development of Immune Sows

Cull sows and naive gilt (unmated sow designated Dookie).

Challenged on numerous occasions, with culture grown M. hyopneumoniae and lung homogenate. Given intranasally and intratracheally. Period of challenge - from September, 1991 to 21st January, 1992.

Tiamulin antibiotic given 31st January, 1992 to 4th February, 1992. Rested for approximately 8 weeks.

Infectious Challenge

120 ml of frozen culture of M. hyopneumoniae strain Beaufort spun down (12,000 xg, 20 min.) and resuspended in 50 ml complete medium and cultured overnight at 37°C. The overnight culture was centrifuged (12,000 xg, 20 min.) and the Mycoplasma cells resuspended in 10 ml serum free Mycoplasma culture medium. The 10 ml of concentrated mycoplasma was administered to anaesthetised immune sows via a catheter to ensure the inoculum was placed into the trachea.

Three of four days post-challenge, the sows were killed, and lymph nodes draining the lungs taken - these included the left and right tracheobronchial lymph nodes, and the lymph nodes located at the bifurcation of the trachea.

Antibody probes were prepared from pig lymph nodes and utilised to detect putative protection antigens as described in Australian Patent Application 49035/90 referred to above. Separate cell cultures were obtained from individual lymph nodes. Culture supernatants were harvested after 5 days of culture.

Antigen Preparation

Mycoplasma hyopneumoniae strain Beaufort was cultured in Etheridge media until the pH had dropped to between 6.8 and 7.0. Cells of M. hyopneumoniae were harvested from culture by centrifugation at 12,000 xg for 20 min., washed 4 times with either sterile PBS or 0.25 M NaCl and then the pelleted cells extracted with one of the following.

(i) Sodium dodecyl sulphate (SDS)

The cell pellet was resuspended in 0.2% SDS and extracted for 2 hours at 37°C. Insoluble material was pelleted from the extract at 12,000 xg for 10 min. and the soluble extract run on SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

(ii) Triton X-114

The method of Bordier (J. Bio. Chem. 1981, 255:1604-1605) was used to selectively extract membrane proteins using the non-ionic detergent Triton X-114.

The cell pellet was resuspended in cold PBS to 2 mg/ml protein and a cold pre-condensed solution of TX-114 added to give a final concentration of 1% (v/v) TX-114. Extraction was achieved by incubation overnight at 4°C with gentle mixing. Insoluble material was removed by centrifugation at 12,000 xg for 20 min. at 4°C. The Triton X-114 soluble membrane proteins were then obtained by achieving a phase separation at 37°C.

Proteins soluble in TX-114 phase were precipitated with 80% ethanol in the presence of carrier dextran (80,000 molecular weight) at -70°C overnight. The proteins were collected by centrifugation at 12,000 xg for 30 min. and dissolved to 500 ug/ml in 4 M urea.

Identification of Antigens

Six antigens were identified utilising the above- mentioned technique. The identified antigens were those that were consistently identified by the antibody probes from the immune cultures and the Dookie gilt. The results are summarised in Table 1.

TABLE 1

Molecular Weight (kD)	Characteristics
110-114	SDS Extracted
90-94	SDS Extracted
72-76	Triton X-114 Extracted
60-64**	SDS Extracted. Partitions to aqueous phase of Triton X-114 extract.
52-54	Triton X-114 Extracted
46-48	Triton X-114 Extracted

** Two antigens of approximate molecular weight 62 kD were identified.

Molecular Weight (kD)	Amino Acid Sequence
46-48	48 K N-Terminal: AGXGQTESGSTSDSKPQAETLKHKV
	48 K CNBR F 1: TIYKPDKVLGKVAVEVLRVLIAKKNKASR
	48 K CNBR F 2: AEQAITKLKLEGFDTQ
	48 K CNBR F 3: KNSQNKIIDLSPEG
52-54	52 K N-Terminal. AGXWAKETTKEEKS
	52 K CNBR F 1. AWVTADGTVN
	52 K CNBR F 2: AIVTADGTVNDNKPNQWVRKY
60-64	62 K N-Terminal: MKLAKLLKGFX (N/L)((M/V) IK
60-64	62 K N-Terminal ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

72-75 74 K N-Terminal: AGXLQKNSLLEEVWYLAL

74 K CNBR F 1: AKNFDFAPSIQGYKKIAHEL

74 K CNBR F 2: NLKPEQILQLLG

74 K CNBR F 3: LLKAEXNKXIEEINTXLDN

CNBR - Cyanogen Bromide fragment

X denotes an undetermined amino acid

(A/B) - residue may be A or B

PCR of 48kDa Gene

Polymerase Chain Reaction (PCR) oligonucleotide primers were designed from the amino acid sequences obtained from the N-terminal and internal cyanogen bromide (CNBr) derived peptides. Inosine (I) was substituted at positions of high redundancy. The following primers were used in a standard PCR assay, run on a Bartelt Gene Machine Robotic thermal cycling instrument.

Oligo 48 K CNBr F 1 : ACIAACGACGAGAAGCCICAGGC

T T A A A

Oligo 48 K CNBr F 2 : TTIAGCTTIGTGATIGCCTGCTC

AT A T T

T

Oligo 48 K CNBr F 3 : AGGTCGATGATCTTCCAICC

AA A A T T

T T

The resulting PCR products were visualised on a 1.5% agarose gel, excised, and purified using Prep-a-Gene (BioRad). They were cloned by standard techniques into a dideoxy tailed T-vector (Holton and Graham, Nucleic Acids Research 19, 1156, 1991) and the nucleic acid sequence determined. The PCR product obtained from the reaction using primers F1 and F2 shown above, was of

approximately 810 base pairs and was shown by sequencing to code for the previously determined amino acid sequence of the purified native 46-48kDa protein.

Genomic clone isolation at 48 k gene

The entire 48k gene and 48kDa protein (Figures 6 and 7) has been isolated and sequenced. The gene was obtained from an *M. hyopneumoniae* genomic library made by digesting genomic DNA with the restriction enzyme CLA I and ligating the fragments into the vector pBluescript (Stratagene). The ligated product was then electroporated into *Escherichia coli* strain SURE (Stratagene) and the cells plated on Luria Broth agar plates containing 100 µg/ml Ampicillin (LB-Amp). The library was screened by DNA hybridisation with a polymerase chain reaction (PCR) product specific for the 48 kDa protein. Positive clones were grown in LB-Amp, the cells harvested and the DNA isolated and partially sequenced for confirmation.

The positive clone pC1-2 was entirely sequenced and the protein sequence deduced. This was compared to the protein sequence obtained from the N terminus and Cyanogen Bromide fragments of the 48 kDa protein to show the that the gene encoded the desired protein.

Adjuvant Selection

Young piglets, 5-7 weeks of age, were immunised with identified antigen(s). The antigens include Triton X-114 extract and identified proteins of 46-48, 52-53, 60-64, 70-75, 90-94 and 110-114 kD, either singly or in combination. An immunising dose of antigen, containing between 5-100 µg protein, was given by intramuscular injection in combination with an adjuvant. An adjuvant is selected from:

(i) Seppic Montanide ISA-50

(ii) Quill A and other derivatives of saponin.

(iii) oil in water emulsion employing a mineral oil such as Bayol F/Arlacel A.

(iv) oil in water emulsion employing a vegetable oil such as corn oil

safflower oil or other with lecithin as emulsifier,

(v) aluminium hydroxide gel, and

(vi) nonionic block polymer such as Pluronic F-127 produced by BASF (U.S.A.).

Immunising doses were given at 2-4 week intervals, the number of doses being dependent on the adjuvant and amount of antigen, but preferably 2 to 3 doses are given.

Adjuvants were treated on the basis of being able to induce antibody titres, as measured by ELISA, and by assessment of induced cell-mediated immunity as tested by Delayed-Type Hypersensitivity (DTH) reaction.

The results clearly show that mineral-oil type adjuvants are consistently superior at inducing antibody titres and DTH responses (Table 2). In particular an adjuvant marketed under trade designation Montanide ISA-50 and available from Seppic, Paris, France has been found to be suitable.

TABLE 2

GROUP	Animal Number	DTH 24 Hour Response	DTH 48 Hour Response	Antibody Levels (450 nm)
	19	0	0	0.061
	11	0	0	0.010
CONTROL	1	-	-	0.005
(Unvaccinated)	15	0	0	0.038
	7	0	0	0.005
	18	+	0	0.753
QUIL A	25	+	0	0.788
	17	0	0	0.638
	168	±	±	0.642
	169	+++	0	0.316
	22	0	0	0.621
VEG. OIL	4	+	0	0.666
	6	+	-	0.239
	13	+-+	++	0.457
	14	+++	++	1.086
	5	+++	++	1.024
MIN. OIL	23	+++	+	0.864
	16	+++	0	0.975
	21	+	±	0.954

TABLE 2. Antibody levels and DTH responses in pigs measured 2 weeks after the third injection of antigen from M. hyopneumoniae. (- = no response; ± = faint reddening; + = faint reddening and swelling; ++ = reddening; +++ = swelling with or without reddening).

Protection Pen Trial

Groups of 9 young piglets, 6 weeks of age, were immunised with purified and semi-purified antigens as shown in Table 3 below. The antigens were purified on reversed-phase HPLC using a formic acid solvent system with an acetonitrile gradient.

Antigens were resolubilised in 4 Molar urea before incorporation in mineral oil adjuvant.

The immunisation schedule is as shown in Table 2.

TABLE 3

Protocol for Pen Trial of Antigens of Mycoplasma Hyopneumoniae

VACCINATIONS & BLEEDS

Treatment	Day Number
1st Vaccination	0
2nd Vaccination	14
3rd Vaccination	50
Infectious Challenge	64
Slaughter	91

ANTIGEN DOSES

Partly Purified 62 kD	1st & 2nd Vaccns. 50µg COMPLEX ANTIGEN/DOSE 3rd Vaccn. - 220µg PARTIALLY PURIFIED ANTIGEN/DOSE
(Purified)74÷52kD	1st Vaccn. 20µg total protein/DOSE 2nd Vaccn. 13µg total protein/DOSE 3rd Vaccn. 17µg total protein/DOSE
(Purified) 46KD	1st Vaccn. 20µg/DOSE 2nd Vaccn. 18µg/DOSE 3rd Vaccn. 27µg/DOSE

ALL PROTEIN ESTIMATIONS DONE BY "BCA" PROTEIN ASSAY (Pierce, Illinois, U.S.A.

Protection from infection with Mycoplasma hyopneumoniae was assessed by infectious challenge 2 weeks after the final immunisation. Infectious challenge was achieved by intranasal administration of 10ml of a 10% (w/v) lung homogenate, prepared from infected lung, and by housing test piglets with

previously infected piglets. Four weeks after infectious challenge, the animals were killed and the extent and degree of lung lesions assessed (Table 4).

TABLE 4

Pen Trial of Antigens of Mycoplasma Hyopneumoniae

Group No.	No. Pneumonia Free (%)	Median Lung Lesion Score	% Reduction (from Median)
Controls	1 (11)	13	0%
62 kD	0 (0)	5	61%
74÷52 kD	3 (33)	6.75	48%
48 kD	2 (22)	6.25	52%

REFERENCE

Warren H.S. and Chedid, L.A., Future Prospects for Vaccine Adjuvants CRC Critical Reviews in Immunology 8 : 83-108, 1988.

Finally, it is to be understood that various other modifications and/or alterations may be made without departing from the spirit of the present invention as outlined herein.

CLAIMS:

1. A putative protective antigen against a Mycoplasma, prepared by a method including

providing

a sample of a Mycoplasma;

an antibody probe including at least one antibody against a Mycoplasma produced by a method including;

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

2. A putative protective antigen according to claim 1 wherein the Mycoplasma is Mycoplasma hyopneumoniae.

3. A putative protective antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), as herein described, mutants, derivatives and fragments thereof.

4. A putative protective antigen according to claim 3 which is a surface protein.

5. A putative protective antigen according to claim 3 or 4 which is a surface lipo-protein or membrane protein.

6 A putative protective antigen according to any one of claims 3-5 having approximate molecular weight of 110-114, 90-94, 74, 62, 52 and 48 kD.

7. A putative protective antigen according to claim 3 wherein the antigen in the 72-75 kD region contains the following N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

8. A putative protective antigen according to claim 7 further including one or more of the following N-terminal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLDN

9. A putative protective antigen according to claim 3 wherein the antigen in the 60-64 kD region contains the following N-terminal amino acid sequence:

MKLAKLLKGFX(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

10. A putative protective antigen according to claim 3 wherein the antigen in the 52-54 kD region contains the following N-terminal amino acid sequence:

AGXWAKETTKEEKS

11. A putative protective antigen according to claim 10 further including one or more of the following N-terminal amino sequences:

AWVTADGTVN

AIVTADGTVNDNKPNQWVRKY

12 A putative protective antigen according to claim 3 wherein the antigen in the 46-48 kD region contains the following N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

13. A putative protective antigen according to claim 12 further including one or more of the following internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR

AEQAITKLKLEGFDTQ

KNSQNKIIDLSPEG

14. An isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment including the following sequence, mutants, derivatives, recombinants and fragments thereof:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550

TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1500
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1550
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782

15. An isolated nucleic acid fragment according to claim 14 encoding a putative protective antigen wherein the antigen is in the 46-48 kD region including the following nucleic acid sequence, mutants, derivatives, recombinants and fragments thereof:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTTATCT	ATTTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGTAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCCTG	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCCAAG	AGACAATTTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TTCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCCAGG	ATGAAATTAT	GGAACTGCCG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAAATA	TGACAATTTA	1550
TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAGTCGA	AAACGAACTA	1650
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTTA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782

16. A method for producing an antibody against a Mycoplasma including

providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion;

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells.

17. A method according to claim 16 wherein the biological sample is taken at a predetermined time after the animal has been challenged with a Mycoplasma, preferably 2 to 7 days after challenge.

18. A method according to claim 16 wherein the culturing of cells in vitro further includes addition of helper factors to the culture, said helper factors selected from the group including cytokines used alone or in combination, including Interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.

19. A method according to any one of claims 16-18 further including a cell activation step including activating the cells isolated to proliferate and secrete and/or release antibodies

said cell activation step including adding a cell activating agent to the culture medium, said cell activating agent selected from the group including mitogens as herein described and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.

20. A method according to any one of claims 16-19 wherein the antibody is in the form of the supernatant harvested from the culture medium.

21. An antibody against a Mycoplasma prepared according to the method of any one of claims 16-20

22. A method of identifying a putative protective antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae, said method including providing

a sample of a Mycoplasma; and

an antibody probe including at least one antibody against a Mycoplasma;

probing the Mycoplasma sample with the antibody probe to detect at least one antigen; and

isolating the antigen detected.

23. A method of purifying a putative protective antigen associated with a Mycoplasma, preferably Mycoplasma hyopneumoniae, said method including providing

a crude antigen mixture; and

an antibody against a Mycoplasma immobilized on a suitable support;

subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and

isolating the purified antigen so formed.

24. A method for preparing a synthetic antigenic polypeptide against Mycoplasma, preferably Mycoplasma hyopneumoniae, which method includes providing

a cDNA library or genomic library derived from a sample of Mycoplasma; and

an antibody probe including an antibody prepared according to claim 16;

generating synthetic polypeptides from the cDNA library or genomic library,

probing the synthetic polypeptides with the antibody probe, and

isolating the synthetic antigenic polypeptide detected thereby.

25. A method according to claim 24 wherein the antibody probe includes an antibody raised against an antigen against Mycoplasma hyopneumoniae, or related infections, selected from the group of antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons (kD), as herein described, mutants, derivatives and fragments thereof.

26. A synthetic putative protective antigen in the 72-75 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

AGXLQKNSLLEEVWYLAL

27. A synthetic putative protective antigen according to claim 26 further including internal amino acid sequences:

AKNFDFAPSIQGYKKIAHEL

NLKPEQILQLLG

LLKAEXNKXIEEINTXLDN

28. A synthetic putative protective antigen in the 60-64 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

MKLAKLLKGFX(N/L)(M/V)IK

ADP(F/I)(R/E)Y(V/A)PQG(Q/A)X(M/N)VG

29. A synthetic putative protective antigen in the 52-54 kD region produced by a method according to claim 24 or 25 having an n-terminal amino acid sequence:

AGXWAKETTKEEKS

30. A synthetic putative protective antigen according to claim 29 further including internal amino acid sequences.

AWVTADGTVN

AIVTADGTVNDNKPNQWVRKY

31. A synthetic putative protective antigen in the 46-48 kD region produced by a method according to claim 24 or 25 having an N-terminal amino acid sequence:

AGXGQTESGSTSDSKPQAETLKHKV

32. A synthetic putative protective antigen according to claim 31 further including internal amino acid sequences:

TIYKPDKVLGKVAVEVLRVLIAKKNKASR

AEQAITKLKLEGFDTQ

KNSQNKIIDLSPEG

33. A vaccine or veterinary composition including a prophylactically effective amount of at least one putative protective antigen against a Mycoplasma according to any one of claims 1-13.

34. A vaccine or veterinary composition according to claim 33 including a plurality of putative protective antigens selected from antigens having approximate molecular weights of 110-114, 90-94, 72-75, 60-64, 52-54 and 46-48 kilodaltons.

35. A vaccine or veterinary composition including an antibody against a Mycoplasma according to claim 21.

36. A diagnostic kit including a diagnostic antigen or fragment thereof according to any one of claims 1-13 and 26-32.

37. A method for preventing or treating a Mycoplasma infection, which method including administering to an animal a prophylactically or therapeutically effective amount of at least one putative protective antigen according to any one of claims 1-13.

38. An isolated DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragment having a nucleic acid sequence according to Figure 6 or an homologous sequence, and functionally active fragments, mutant, variant or recombinant thereof.

39. A clone including a DNA fragment according to claim 38.

40. A clone according to claim 39 which is clone pC1-2 as hereinbefore described.

41. An amino acid sequence or functional equivalent thereof encoded by the DNA fragment according to claim 38.

42. An amino acid sequence or functional equivalent thereof having the amino acid sequence of Figure 7

43. A putative protective antigen or antibody substantially as hereinbefore described with reference to the examples.

08/913430

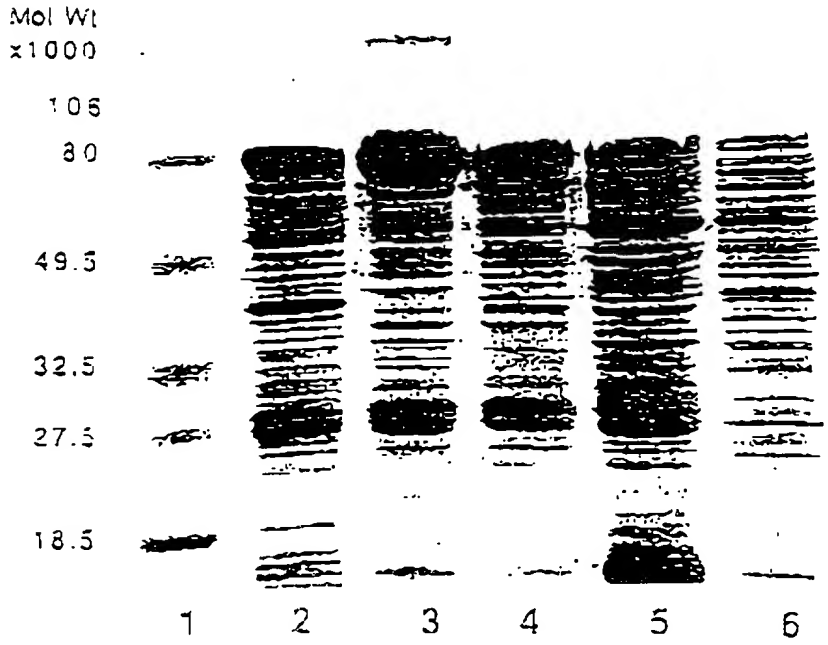

FIG. 1

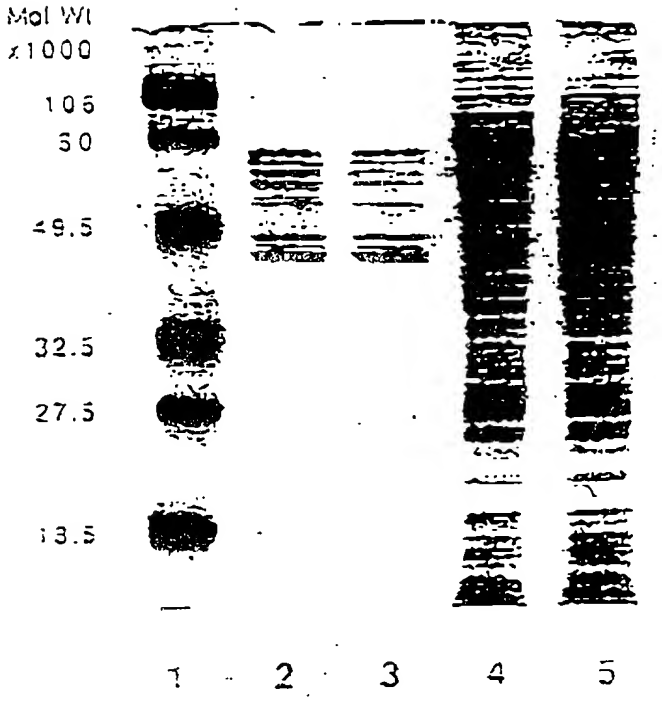

FIG. 2

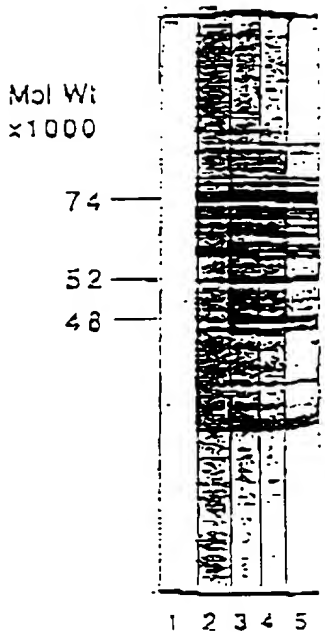

FIG. 3

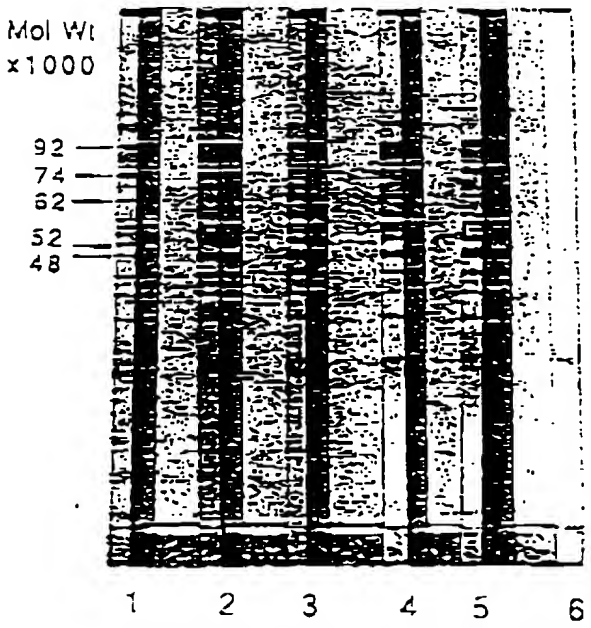

FIG. 4

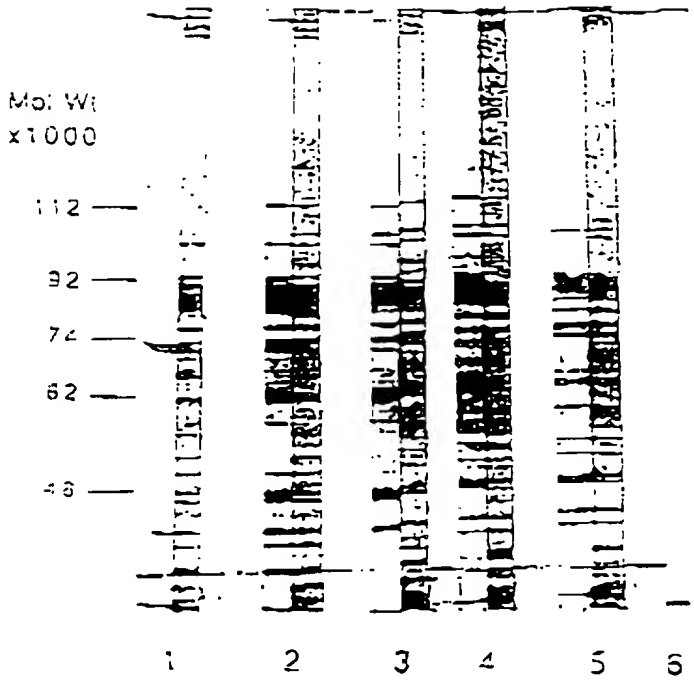

FIG. 5

```
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AAAAAGTAGA ACAAAAGTTA TTAATGTAAA CATTAGCGCA ATCCTTAAGA     150
AAAAATTAAA AGTTTTATCT ATTTTTTTTA ATCGAAATCC AACCAGGCAT     200
AAATCTTTGT CAGTATTTAT CAAGTCGGTA TTTTTTCATT ATTTCTACTA     250
AAATATTATT TGAATTTGCA TTTTCCATAA TCTAAAATTT TACATTTTTT     300
TATAACAATT TTTAAAAATT ACTCTTTAAT TTATAGTATT TTTTTATTTT     350
TTAGTCTAAA TTATAAAATT ATCTTGAATT TTATTTGAAT TTTTATAATT     400
TAGTACTAAA AAATACAAAT ATTTTTTCCT ATTCTAAGAA AAATTCATTT     450
TTTAAAAAAA ATTGATTTTT ATAGTATAAT TTGTTTGTAT AATTGAATTA     500
ACTTGATTTG AAAGGGAACA AAATGAAAAA AATGCTTAGA AAAAAATTCT     550
TGTATTCATC AGCTATTTAT GCAACTTCGC TTGCATCAAT TATTGCATTT     600
GTTGCAGCAG GTTGTGGACA GACAGAATCA GGTTCAACTT CTGATTCTAA     650
ACCACAAGCC GAGACGCTAA AACATAAAGT AAGTAATGAT TCTATTCGAA     700
TAGCACTAAC CGATCCGGAT AATCCTCGAT GAATTAGTGC CCAAAAAGAT     750
ATTATTTCTT ATGTTGATGA AACAGAGGCA GCAACTTCAA CAATTACAAA     800
AAACCAGGAT GCACAAAATA ACTGACTCAC TCAGCAAGCT AATTTAAGCC     850
CAGCGCCAAA AGGATTTATT ATTGCCCCTG AAAATGGAAG TGGAGTTGGA     900
ACTGCTGTTA ATACAATTGC TGATAAAGGA ATTCCGATTG TTGCCTATGA     950
TCGACTAATT ACTGGATCTG ATAAATATGA TTGGTATGTT TCTTTTGATA    1000
ATGAAAAAGT TGGTGAATTA CAAGGTCTTT CACTTGCTGC GGGTCTATTA    1050
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CCCAAGATGA TAATAATTCC CAATATTTTT ATAATGGTGC AATGAAAGTA    1200
CTTAAAGAAT TAATGAAAAA TTCGCAAAAT AAAATAATTG ATTTATCTCC    1250
TGAAGCGGAA AATGCTGTTT ATGTCCCAGG ATGAAATTAT GGAACTGCCG    1300
GTCAAAGAAT CCAATCTTTT CTAACAATTA ACAAAGATCC AGCAGGTGGT    1350
AATAAAATCA AAGCTGTTGG TTCAAAACCA GCTTCTATTT TCAAAGGATT    1400
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GATAAAGCCA AAACTTTTAT CAAAGACGGC GATCAAAATA TGACAATTTA    1550
TAAACCTGAT AAAGTTTTAG GAAAAGTTGC TGTTGAAGTT CTTCGGGTTT    1600
TAATTGCAAA GAAAAATAAA GCATCTAGAT CAGAAGTCGA AAACGAACTA    1650
AAAGCAAAAC TACCAAATAT TTCATTTAAA TATGATAATC AAACATATAA    1700
AGTACAAGGT AAAAATATTA ATACAATTTT AGTAAGTCGA GTAATTGTTA    1750
CAAAAGCTAA TGTTGATAAT CCTGATGCCT AA                       1782
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FIG. 6

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          10         20         30         40         50
1234567890 1234567890 1234567890 1234567890 1234567890

MKKQLPKKFL YSSAIYATSL ASIIAFVAAG CGQTESGSTS DSKPQAETLK      50
HKVSNDSIRI ALTDPDNPRW ISAQKDIISY VDETEAATST ITKNQDAQNN     100
WLTQQANLSP APKGFIIAPE NGSGVGTAVN TIADKGIPIV AYDRLITGSD     150
KYDWYVSFDN EKVGELQGLS LAAGLLGKED GAFDSIDQNN EYLKSHMPQE     200
TISFYTIAGS QDDNNSQYFY NGANKVLKEL NGNSQNKIID LSPEGENAVY     250
VPGWNYGTAG QRIQSFLTIN KDPAGGNKIK AVGSKPASIF KGFLAPNDGM     300
AEQAITKLKL EGFDTQKIFV TRQDYNDKAK TFIKDGDQNM TIYKPDKVLG     350
KVAVEVLRVL IAKGNKASRS EVENELKAKL PNISFKYDNQ TYKVQGKNIN     400
TILVSPVIVT KANVDNPDA                                       419
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FIG. 7

Attorney's Docket No. U 011415-0 **PATENT**

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type: (check one applicable item below)

- ☐ original
- ☐ design
- ☐ supplemental

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

- ☒ national stage of PCT

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

- ☐ divisional
- ☐ continuation
- ☐ continuation-in-part (C-I-P)

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

ANTIGEN COMPOSITION AGAINST MYCOPLASMA

SPECIFICATION IDENTIFICATION

the specification of which: *(complete (a), (b) or (c))*

(a) ☐ is attached hereto.

(b) ☐ was filed on _____ as ☐ Serial No. 0 /_____
or ☐ Express Mail No., *as Serial No. not yet known* _____
and was amended on _____ *(if applicable)*.

NOTE: Amendments filed after the original papers are deposited with the PTO which contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 CFR 1.67.

(c) ☒ was described and claimed in PCT International Application No. PCT/AU96/00149 filed on 15 March 1996 and as amended under PCT Article 19 on _____ (*if any*).

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information

☒ which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56

(also check the following items, if desired)

☐ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☐ In compliance with this duty there is attached an information disclosure statement in accordance with 37 CFR 1.98.

PRIORITY CLAIM (35 U.S.C. § 119)

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☐ no such applications have been filed.

(e) ☒ such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.

A. PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
AU	PN 1789	16 March 1995	☒ YES NO ☐
PCT	PCT/AU96/00149	15 March 1996	☒ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

NOTE: *If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.*

POWER OF ATTORNEY

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (*List name and registration number*)

13

PAUL B. WEST, ~~18947~~
JOSEPH H. HANDELMAN, 26179
JOHN RICHARDS, 31053
JOHN J. CRYSTAL, ~~26360~~
RICHARD J. STREIT, ~~25765~~
ALAN K. ROBERTS, ~~17777~~
S. DELVALLE GOLDSMITH, 14383
PETER D. GALLOWAY, ~~27885~~
IAIN C. BAILLIE, 24090
THOMAS F. PETERSON, 24790
RICHARD P. BERG, 28145
JULIAN H. COHEN, 20302
WILLIAM R. EVANS, 25858

(check the following item, if applicable)

☐ Attached as part of this declaration and power of attorney is the authorization of the above-named attorney(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO

LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

(212)708-1930

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: *Carefully indicate the family (or last) name as it should appear on the filing receipt and all other documents.*

Full name of sole or first inventor

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Inventor's signature [signature]

Date 6/10/97 **Country of Citizenship** Australia

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Rogan *(GIVEN NAME)* | *(MIDDLE INITIAL OR NAME)* | LEE *FAMILY (OR LAST NAME)*

Inventor's signature Rogan Lee

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Full name of third joint inventor, if any

Stephen (GIVEN NAME) William (MIDDLE INITIAL OR NAME) DOUGHTY (FAMILY (OR LAST NAME))

Inventor's signature [signature]

Date 25/9/97 **Country of Citizenship** Australia

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Post Office Address 1A Diana Drive, Blackburn, Victoria 3130 Australia

[signature]

CHECK PROPER BOX(ES) FOR ANY OF THE FOLLOWING ADDED PAGE(S) WHICH FORM A PART OF THIS DECLARATION

☐ Signature for fourth and subsequent joint inventors. *Number of pages added* _____

• • •

☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____.

• • •

☐ Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

• • •

☐ Added page for signature by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time (37 CFR 1.47).

• • •

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____

• • •

☐ Authorization of attorney(s) to accept and follow instructions from representative.

• • •

(If no further pages form a part of this Declaration, then end this Declaration with this page and check the following item:)

☒ This declaration ends with this page.